Role of the tumor microenvironment in the lymphatic metastasis of cervical cancer (Review)

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Abstract. Lymphatic metastasis is the primary type of cervical cancer metastasis and is associated with an extremely poor prognosis in patients. The tumor microenvironment primarily includes cancer-associated fibroblasts, tumor-associated macrophages, myeloid-derived suppressor cells, immune and inflammatory cells, and blood and lymphatic vascular networks, which can promote the establishment of lymphatic metastatic sites within immunosuppressive microenvironments or promote lymphatic metastasis by stimulating lymphangiogenesis and epithelial-mesenchymal transformation. As the most important feature of the tumor microenvironment, hypoxia plays an essential role in lymph node metastasis. In this review, the known mechanisms of hypoxia, and the involvement of stromal components and immune inflammatory cells in the tumor microenvironment of lymphatic metastasis of cervical cancer are discussed. Additionally, a summary of the clinical trials targeting the tumor microenvironment for the treatment of cervical cancer is provided, emphasizing the potential and challenges of immunotherapy.

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Abbreviations: CC, cervical cancer; CAF, cancer associated fibroblast; TAM, tumor associated macrophage; CSF, colony stimulating factor; MDSC, myeloid-derived suppressor cell; EMT, epithelial-mesenchymal transformation; TME, tumor microenvironment; PGE2, prostaglandin E2; VEGF, endothelial growth factor; LEC, lymphatic endothelial cell; LEC, lymphatic endothelial cell; LN, lymph node; LNM, LN metastasis; HIF, hypoxia inducible factor; TIME, humor immune microenvironment

Key words: lymphatic metastasis, tumor microenvironment, cervical cancer, cancer-associated fibroblast, tumor-associated macrophage

Contents

1. Introduction

Cervical cancer (CC), as one of the most common causes of female mortality, poses a serious threat to women's lives and health. Globally, in 2020, there were an estimated 604,127 CC cases and 341,831 related deaths, with a corresponding age-standardized incidence of 13.3 cases per 100,000 women-years and a mortality rate of 7.2 deaths per 100,000 women-years (1). Lymph node metastasis (LNM) is the most common type of CC metastasis and is closely related to prognosis. The more extensive the LNM is, the worse the prognosis of patients. Studies have confirmed that the overall 5-year survival rates of CC patients with 0, 1-2, 3-9 and 10 or more metastatic lymph nodes are 90, 69, 57 and 35%, respectively (2). According to the 2009 FIGO staging principle, LNM does not affect the International Federation of Obstetrics and Gynecology (FIGO) CC staging. However, the FIGO staging system released in 2018 clearly states that once CC patients are diagnosed with LNM, they can be directly diagnosed with stage IIIC or above CC, which fully demonstrates the important role of LNM in the progression of CC (3). Unfortunately, little is known regarding the LNM mechanism in CC, which remains one of the biggest challenges in treating CC (4).

The tumor microenvironment (TME) is primarily composed of fibroblasts, endothelial cells, different subsets of infiltrating immune cells (IICs), bone marrow-derived progenitor cells, platelets, and inflammatory cytokines (5). Previous studies have confirmed that tumor cells or host-derived cells (immune cells and fibroblasts, amongst others) in the TME can release various lymphatic angiogenic factors, such as vascular endothelial growth factor (VEGF)-A, C and D, lymphatic vascular factor angiogenin-2, and hepatocyte growth factor,

which can stimulate angiogenesis and lymphangiogenesis (6). Tumor cells, tumor stromal cells, and infiltrating white blood cells release chemokines to recruit different immune cell types into the TME. Chemokines can be grouped into four main classes, depending on the location of the first two cysteine (C) residues of their primary protein structure, namely, the C, CC, CXC and CX3C chemokines. All chemokines signal by binding to cognate heterotrimeric G protein-coupled receptors (GPCRs) of the rhodopsin-like family found on migratory cells (7). According to the special needs of migration in each environment, chemokines can act as tumor angiogenesis media, directly interact with chemokine receptors on endothelial cells, and induce tumors to promote the release of growth factors. These growth factors can promote tumor growth in a paracrine signaling manner, thus improving migration, proliferation, and endothelial cell survival. In addition, chemokines can also cooperate with other angiogenesis promoters. For example, VEGF-, CXCL8- and CXCL12-induced upregulation of VEGF expression produces a positive feedback effect, and VEGF further stimulates the production of angiogenic chemokines (8). In addition, lymphatic endothelial cells (LECs) in tumor-draining lymph nodes have been proven to proliferate, leading to the expansion of the lymphatic sinus (9). Hypoxia can stimulate the formation of lymphatic vessels (lymphangiogenesis) and blood (angiogenesis), such that cancer cells can escape from the unfavorable tumor microenvironment and spread to an environment conducive to its survival, ultimately leading to metastatic diseases and mortality (10). Currently, research has confirmed that hypoxia promotes lymphatic metastasis primarily through HIF-1α promoting VEGF-A/-C/-D, TGF-β transcriptional activation of lymphatic vessel generation mediated by signal cascades such as Prox-1. Multiple factors, such as ET-1, C/EBP- δ, EGR-1, AP-1, MIF and NF-κB, can also promote lymphatic metastasis by promoting the proliferation and migration of LECs (11).

In the present review, the known mechanisms of hypoxia, and the involvement of stromal components and immune inflammatory cells in the tumor microenvironment in lymphatic metastasis of CC is discussed, and a summary of the clinical trials for strategies targeting the tumor microenvironment for the treatment of CC is provided.

2. Mechanism of lymphatic metastasis in cancer

Tumor cell entry into the lymphatic vasculature is the first step of metastasis. The lymphatic system primarily regulates fluid homeostasis and the immune response. Lymphatic metastasis plays an active role in the spread and metastasis of primary tumors (12). Similar to angiogenesis, lymphangiogenesis is a multi-step process. On the one hand, activated LECs proliferate and migrate under specific stimuli to form new blood vessels; on the other hand, cancer cells invading the afferent lymphatic vessels spread to the tumor-draining lymph nodes, which are an important hub for the stagnation and growth of metastatic cells, immune regulation, and secondary dissemination to distant sites (13). The process of tumor cells entering lymphatic vessels primarily includes the following steps: i) Cancer cell invasion through the basement membrane; ii) tumor-associated lymphoid hyperplasia; iii) tumor cell recruitment and clustering around lymphatic vessels; iv) secretion of cytokines by lymph endothelial cells to change the microenvironment and cause immune escape; and v) tumor cell entry into lymphatic vessels. The primary mechanisms of the last method include: i) Mechanical destruction of the lymphatic endothelial wall; ii) infiltration dependent on the CCL21 concentration gradient and via the lymphatic endothelial valve; iii) increased lymphatic permeability induced by mechanisms such as upregulation of α4β1 integrin and its ligand VCAM-1 in LECs; iv) release of chemicals to induce contraction of LECs to form an invasion site. The specific mechanisms are shown in Fig. 1.

Cancer cell invasion through the basement membrane. Tumor cells at the invasion front usually show infiltrative behaviors and penetrate into surrounding tissues in the form of cell stripes or clusters or individual cells, and this process is primarily mediated by epithelial to mesenchymal transformation (EMT) in cancer cells, which is an evolutionarily conserved cell process and is critical to embryogenesis and pathological reactions (such as wound healing or tissue repair) (14). EMT mainly occurs by activating Wnt or TGF-β signal transduction, hypoxia, and inflammation-related pathways, further inducing the expression of several key transcription factors of the twist, Snai1 and Zeb families in the TME (15). In turn, these transcription factors mediate several phenotypic changes in cancer cells; they mediate the downregulation of epithelial traits (including cell polarity and cell connectivity) and induction of mesenchymal characteristics, such as cytoskeleton remodeling and the expression of extracellular matrix (ECM)-degrading proteases, allowing cells to invade surrounding tissue effortlessly. It is worth noting that EMT is a gradual process and may occur throughout the entire process of tumor progression (16).

Tumor-associated lymphangiogenesis. Research has revealed that increased expression of the lymphatic angiogenic factors VEGF-C and VEGF-D can significantly promote LNM in esophageal squamous cell carcinoma (17). Compared with nonmetastatic tumors, metastatic melanoma is characterized by increased lymphangiogenesis, and the degree of tumor lymphangiogenesis is an important indicator for predicting the overall survival rate and LNM in patients (18). Tumor lymphangiogenesis and VEGF-C expression can serve as indicators of sentinel LNM during surgical resection of primary melanoma (19). In addition to increasing the quality of tumor-related lymphatic vessels, lymphangiogenic factors can also increase the expression of chemokines or adhesion molecules and receptors involved in tumor cell-LEC interactions by activating lymphatic endothelial cells, thus actively promoting cancer dissemination (20). In general, lymphatic hyperplasia occurs first in tumor LNM. The increased number and size of peripheral lymphatic vessels may provide more opportunities for cancer cells to infiltrate (lymph infiltration), but tumor drainage lymphatic vessels may also promote tumor proliferation by increasing lymphatic flow and drainage mediated by VEGF-C (21).

Tumor cells are attracted to and cluster around lymphatic vessels. Lymphatic hyperplasia serves as a prerequisite for LNM, which primarily involves cancer cell migration to lymphatic vessels and recruitment of cancer cells and supporting cells to the lymph nodes, providing a very

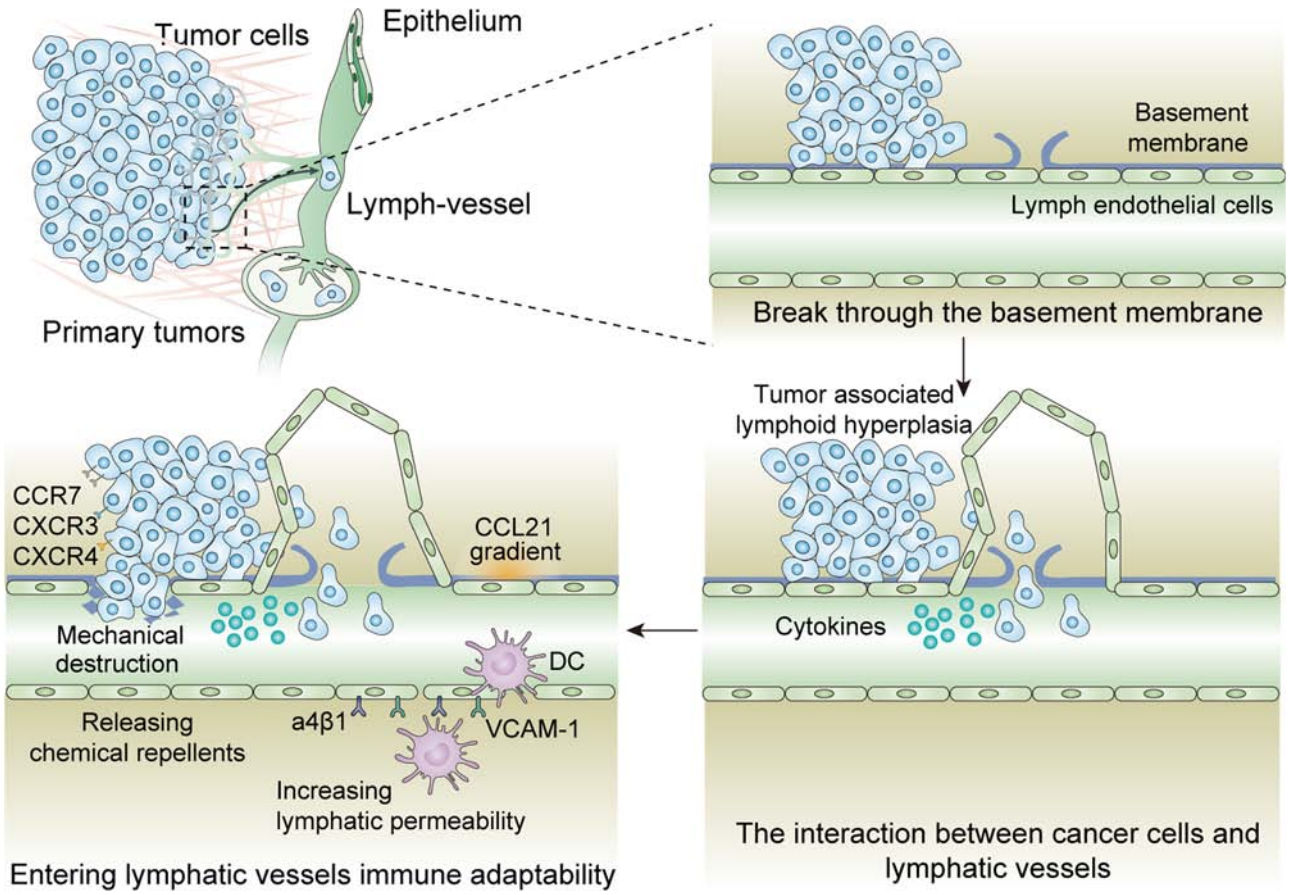

Figure 1. The process and mechanism of lymphatic metastasis.

conducive tumor microenvironment for cancer stem cells; this microenvironment regulates the antitumor immune response at the level of primary tumors and metastatic lymph nodes. In most normal tissues, lymphatic vessels can secrete a large amount of the chemokine CCL21, which can enter the lymphatic system by binding to the activated CCR7 receptor on DCs and can ultimately be excreted from lymph nodes to initiate an *in vivo* immune response (22). *In vitro* studies have confirmed that enhanced lymphatic flow can increase CCL21 production in the lymphatic endothelium (23). Importantly, when CCR7 was overexpressed in transplanted melanoma cells, LNM was greatly enhanced in an experimental tumor model, which indicates that LECs can serve as guiding clues for metastatic cancer cells in the physiological function of the immune system (24).

Chemokine CXCL12 (matrix-derived factor 1) was reported for the first time as a dependent factor involved in the *in vivo* lymphatic metastasis of Paget's disease (25). CXCL12 is upregulated in the lymphatic vascular endothelium of the subcapsular sinus of tumor-draining lymph nodes and tumor-associated lymphatic endothelial cells, while its receptor CXCR4 is expressed by invading tumor cells (26). In addition, the expression of CXCR3 in tumor cells is also associated with an increased LNM rate (27). CCL1 chemokines produced by the lymphatic sinuses mediate the entry of melanoma cells expressing CCR8 into the lymph nodes while blocking CCR8 suppresses LN metastasis. In particular, CCR8 inhibition leads to the stagnation of tumor cells in clusters of lymphatic vessels at the junction with the subcapsular LN sinus (28).

Tumor cell intravasation into lymphatic vessels. Previous studies have confirmed that tumor cells enter lymphatic vessels primarily via one of four ways: i) Mechanical injury or destruction of the lymphatic endothelial wall; ii) by detection of a CCL21 gradient and infiltrating through the junctions of the lymphatic endothelial valves; iii) by increasing lymphatic permeability, such as through cell growth factor (TGF-β1)-induced upregulation of α4β1 integrin and its ligand VCAM-1 in LECs; and iv) LEC contraction and formation of the invasion gate (CCID) induced by the release of chemical repellents [such as 12 (s)-hete] (29). Additionally, research has confirmed that CCR7+ cancer cells can escape the primary tumor and drift to the tumor-draining lymph nodes by increasing the expression of CCL21 in tumor-related lymphatic vessels via VEGF-C (30).

Lymph endothelial cells secrete cytokines to alter the microenvironment to facilitate immune escape. Lymph endothelium can not only provide a stable microenvironment for a cancer with stem cell-like characteristics but also provides a protective environment for long-term survival and subsequent metastasis of tumor cells. In addition, tumor cells may remain dormant in draining lymph nodes for a long period of time after primary tumor resection (31). The clinical observation of so-called 'transit metastasis' (that is, a metastatic tumor that

has developed in the lymphatic vessel between the draining LN and the primary tumor) revealed that CXCR4 melanoma cells (CD133+ melanoma cells) were located near the lymphatic vessel-producing CXCL12 in the metastatic lymph nodes and lungs. The metastatic activity of CXCR4+/CD133+ cells was higher than that of CXCR4-/CD13- cells. The study also confirmed that the combined use of the alkylating agent dacarbazine and CXCR4 blocker, which are widely used to treat human melanoma, is significantly better than the single use of dacarbazine in inhibiting the growth, migration, and metastasis of melanoma (32). In addition to these direct effects of LECs on the survival of cancer cells, lymphatic vessels may also provide an immunoprotective microenvironment through the secretion of chemokines (33). Studies have shown that CCL21 may transform the host immune response from immunogenicity to tolerance, which may potentially promote tumor progression (34). It has been reported that LN lymphatic vessels activated by VEGF-C not only promote tumor metastasis of melanoma but also induce immune tolerance, which increases the difficulty of treatment (35). Recently, study revealed that LECs induce tolerance via programmed cell death 1 (PD1) ligand 1 (PD-L1) and lack of costimulation leading to high-level PD-1 expression on CD8 T cells (36). Therefore, the activities and interactions of various cells in the TME play a decisive role in lymphatic metastasis.

3. Role of the tumor microenvironment in the lymphatic metastasis of CC

Characteristics of the tumor microenvironment in CC. The unique tumor microenvironment of CC is primarily estrogen receptor α (ERα) matrix activation, sustained high-risk human papillomavirus infection, hypoxia, and matrix and immune inflammatory cells (mainly including CAFs, TAMs and MDSCs), T cells, and neutrophils, which ultimately promote angiogenesis and inflammation (37,38). First, 17-β-estradiol (E2) interacts with the matrix ERα on the surface of myofibroblasts and fibroblasts and further induces an increase in the secretion of anti-apoptotic factors, inflammatory chemokines, extracellular matrix enzymes, and proangiogenic factors. In addition, epithelial cells with persistent high-risk HPV infection can attract monocytes (monocyte chemoattractant protein-1 and macrophage inflammatory protein-3α), natural killer cells, and Th17 lymphocytes. Positive expression of cancer proteins (E6 and E7) in high-risk HPV strains can trigger a series of events to promote CC metastasis through their host cervical epithelial cells. In short, in contrast to the matrix progesterone cascade mediated by the progesterone receptor (PR), the synergistic effect between the activity of stromal ERα and high-risk HPV oncoproteins induces CC proliferation and promotes inflammation and angiogenesis, participating in mesenchymal-epithelial and EMT changes (39,40).

In CC, the lymphatic vessels in the diffusion area of tumor cells are in a dynamic process of change, and the lymphatic changes that promote tumor metastasis play a leading role in solid tumor metastasis (41). The biggest obstacle for tumor cells to invade lymphatic vessels is the intact lymphatic vessel structure, which makes it difficult for them to break through. The integrity of lymphatic vessels is mainly related to proteins in the adhesion links between LECs (42). In addition, the complete lymphatic endothelial barrier and sound repair function also depend on the interstitial cells and their secreted cytokines in the surrounding environment (43). The stromal cells and immunoinflammatory cells in the TME can secrete certain cytokines, such as VEGF-C/D, to promote the formation of lymphatic vessels, induce the inactivation of CD4+ and CD8+ T cells, help tumor cells escape immune surveillance, induce tumor cell EMT and enhance tumor cell invasive ability, finally leading to lymphatic metastasis of CC (44). Studies have confirmed that hypoxia can induce the enhancement of the invasive capacity of CC cells and foster a tumor-supporting environment through increased CCL8 secretion and TAM recruitment to promote lymphatic vessel entry and angiogenesis (38). In this review, the mechanisms of hypoxia, and the involvement of stromal components and immune inflammatory cells in the tumor microenvironment in the lymphatic metastasis of CC is summarized.

The role of hypoxia in the lymphatic metastasis of CC. Hypoxia is a key factor that has been identified to lead to a poor prognosis in patients with prostate cancer, pancreatic cancer, breast cancer, head and neck cancer, and other types of tumors. Targeting hypoxia is one of the directions of LNM imaging and staging. Therefore, addressing tumor regional hypoxia is an urgent problem to improve cancer prognosis. The main reasons for the formation of a hypoxic tumor microenvironment are imbalances in blood supply and abnormal tumor metabolism. Once the hypoxic microenvironment is formed, the tumor invasive and metastatic abilities are enhanced, and the tumor cells can present features of a dormant state to avoid immune surveillance, resulting in the failure of immunotherapy. In addition, the activation of HIF can induce EMT, which is the first step for tumor cells to break through the basement membrane and metastasize to a distant location (45). In addition, hypoxia has been proven to stimulate molecular changes in cancer cells to facilitate a state of mitotic arrest to make the cells appear dormant. Increasing evidence shows that dormancy is crucial for cancer cell survival; the cells need to delay their invasive behaviors until the distant 'hostile' microenvironment is transformed to enable them to escape from immune surveillance upon treatment (46). However, the underlying mechanisms of cancer cell EMT, progression and escape from apoptosis/necrosis in hypoxic environments remain unclear. Ju *et al* (47) found that CSN8, as a key factor in hypoxia-induced cell dormancy and EMT occurrence, can promote colorectal cancer cells to evade immune surveillance and attack, significantly improving their invasive and metastatic ability. Hsin *et al* (48) found that upregulation of carbonic anhydrase IX can promote EMT, and this phenotype combined with upregulation of PFKFB4 ultimately improves the migration and metastasis of CC cells. Hypoxia induces EMT in CC to facilitate further lymphatic metastasis.

The difficulty in early prediction and research of LNM lies in the lack of highly specific molecular markers, especially markers of lymphatic vessels and blood vessels around tumors, which has led to extremely slow progress in research on LNM for decades. Breakthroughs have been made following the discovery of VEGF-C/D and its specific receptor VEGFR-3; thus, research on lymphatic metastasis is gradually increasing. Sugiura *et al* (49) found that in oral tumors, hypoxia in the

surgical area can lead to an increase in local lymphangiogenesis, accompanied by a significant increase in CD11b+ cell infiltration and LNM. In CC, Cairns and Hill (50) found that acute hypoxia can reduce the volume of the primary tumor lesion in nude mice but significantly increases the number of LNMs. Chaudary *et al* (51) found that hypoxic treatment of CC cells can significantly upregulate VEGFR3 and promote lymphatic metastasis. Downregulation of VEGFR3/VEGFC or the use of VEGFR3/VEGFC blockers can significantly reduce hypoxia-induced tumor lymphatic proliferation and metastasis. These studies further confirm that hypoxic conditions are more conducive to the increase in VEGFR3/VEGFC and promote the lymphatic metastasis of CC. Lee *et al* (52) found that the prognosis of CC patients with LNM is poor. Identifying novel treatment methods based on the expression of CA9 to prevent LNM is expected to significantly improve the prognosis of CC patients. CA9 is currently recognized as one of the most commonly used markers of hypoxia (53,54). Kim *et al* (55) found that extended-field irradiation (EFI) has a significant inhibitory effect on the recurrence of para-aortic lymph nodes in CA9-positive tumor patients, but it is not significant for improving long-term survival. The reason may be related to increased local and distant metastasis rates. Hypoxia promotes LNM by inducing the release of lymphangiogenic factors, such as VEGF-C, from malignant tumor cells to induce lymphatic dilation (lymphangiogenesis) of the primary tumor and draining sentinel LN.

Hypoxia can also lead to lymphatic metastasis through the release of lymphangiogenic active factors and other factors that recruit TAMs to accumulate in lymphatic vessels and form metastatic areas. Chen *et al* (56) found that high expression of ZEB1 under hypoxic conditions can promote tumor metastasis by increasing TAM recruitment and CCL8 secretion. Targeting ZEB1 can improve the prognosis of patients with metastatic tumors by destroying the hypoxic microenvironment. They also showed that hypoxic TAMs near lymphatic vessels are the primary cells producing IL-10, and a sharp increase in IL-10 concentration induces upregulation of Sp1 expression in LECs, promoting lymphatic angiogenesis in tumors (57). They further confirmed that macrophages recruited to the hypoxic microenvironment of CC tend to transform into the M2 phenotype and induce an increase in Nrp-1 in CC. This suggests that reversing the polarization of TAM towards an M2 phenotype and interfering with Nrp-1 represents a novel strategy for improving the hypoxic microenvironment of CC (58). As an important feature of solid tumors, hypoxia accompanies almost the entire process of tumor metastasis. The hypoxia of the tumor increases invasion and metastasis and helps disguise tumor cells as cells in a dormant state to avoid immune monitoring and attack, increasing the risk of chemotherapy and immunotherapy resistance. Targeted hypoxia therapy is an important direction for improving patient prognosis in the future.

The role of CAFs, TAMs, MDSCs, and immunoinflammatory cells in the lymphatic metastasis of CC. In addition to hypoxia, stromal components and inflammatory immune cells also play an essential role in the process of lymphatic metastasis of CC. These cells will change in morphology and function with tumor progression to promote tumor invasion, migration, lymphatic metastasis, and hematogenous migration. Here, a focus on the role of CAFs, TAMs, MDSCs, and immune cells in the lymphatic metastasis of CC is discussed.

CAFs. One of the primary obstacles in tumor cell infiltration through the lymphatic system is the integrity of the lymphatic endothelium, which is closely related to the protein complexes that make up junctions between endothelial cells. In addition, cell homeostasis, and cytokine dynamic balance in the microenvironment around lymphatic vessels are the basis for maintaining the structural integrity and barrier function of the lymphatic endothelium. CAFs have important physiological functions in maintaining the stability and integrity of most tissues. This function is realized during the progression of metastasis and can induce the formation of a metastasis-promoting microenvironment (43).

Activated CAFs can change the components of the ECM to reshape the tumor microenvironment, promote local angiogenesis, tumor cell proliferation, and metastasis and play a role in the formation of chemotherapy resistance by activating multiple signaling pathways and secreting activated cytokines. The signaling of CAFs and tumor cells plays an important role in tumor progression and treatment. The current view is that CAFs and the tumor immune microenvironment (TIME) mainly promote tumor progression. The antitumor components in the TIME and TME are in dynamic balance and are primarily composed of immune cell populations in tumors. CAFs interact with tumor-infiltrating immune cells to form a tumor-immune suppressive microenvironment by secreting growth factors, cytokines, exosomes, chemokines, and other effectors, assisting tumor cell escape from immune surveillance and attack and promoting tumor cell proliferation and distant metastasis (59). Deep investigation of the mechanisms and interactions between CAFs and tumor cells, as well as between CAFs and other immune cells, may provide novel ideas for immunotherapy.

Previous studies have confirmed that TGF-β1-activated CAFs promote breast cancer EMT, invasion, and metastasis by overexpression of FAP-α, and autophagy in breast cancer. Treatment with both the autophagy inhibitor 3-methyladenine and FAP-α knockdown can reverse EMT and eliminate lung metastasis and invasion caused by CAFs, indicating that autophagy and FAP-α in CAFs are prerequisites for the metastasis of breast cancer to the lungs (60). Wang *et al* (61) revealed that epiregulin reprograms CAFs via the JAK2/STAT3 pathway to facilitate oral squamous cell carcinoma invasion. Zhou *et al* (62) found that CAFs induce EMT functional changes in tongue squamous cell carcinoma. In CC, Murata *et al* (63) reconstituted a metastatic TME by co-transplanting CAFs and cancer cells into nude mice to reconstruct the microenvironment of tumor metastasis. It was surprising to find that 40% of nude mice co-transplanted with two kinds of cells had LNMs, while nude mice transplanted with a single cancer cell had no LNMs. They also showed that CC CAFs secreted large quantities of heparin-bound epidermal growth factor (HB-EGF), and the platelet-derived growth factor produced by ME180 cells enhances the expression of CAF HB-EGF, which in turn can significantly promote the proliferation of ME180 cells (64). Xiao *et al* (65) found that overexpression of TGF-β1 and SDF-1 in CAFs promoted colony formation,

growth, and invasion of CC cells, while when cocultured with TGF-β1 and SDF-1 neutralizing antibodies, these phenomena were reversed. CAFs secrete a series of growth factors through interaction with tumor cells, causing tumor cell invasion, migration, and EMT, eventually leading to LNM of CC, as confirmed by *in vivo* experiments.

CAFs, as matrix-supporting cells around LECs, play a supportive role in maintaining the lymphatic endothelial barrier. Before lymphatic metastasis, CAFs can induce LNM by damaging the lymphatic endothelial barrier. Wei *et al* (66) found a new subgroup of CAFs, namely, periostin+ CAFs, which significantly increased infiltration in patients with CC LNM, and the more infiltration there was, the poorer the prognosis was. Further mechanistic research confirmed that periostin+ CAFs activated the integrin FAK/Src-VE cadherin signaling pathway in LECs, disrupted the lymphatic endothelial barrier, allowed cancer cells to enter the lymphatic vessels, and ultimately cause CC LNM. If the FAK/Src-VE cadherin signaling pathway was inhibited, the effect of periostin+ CAFs was weakened. This study highlights a novel approach to the treatment of CC LNMs, identifying potential targets for blocking CAF-dependent metastasis that destroys the lymphatic endothelial barrier and strengthening and consolidating the integrity and stability of the lymphatic endothelial barrier is essential for blocking CAF-dependent LNM. These studies comprehensively show that CAFs can play a key role in CC lymphatic metastasis by impairing lymphatic endothelial barrier function and secreting growth factors and activating related signaling pathways to promote tumor invasion. Targeting CAFs is thus a relatively novel method for preventing CC lymphatic metastasis.

TAMs. TAMs are involved in almost the entire process of tumor occurrence and development. In the early stages of tumor development, the immune system quickly responds, summoning T cells and macrophages to attack and clear tumor cells through killing and phagocytic functions. However, under certain conditions, macrophages can be easily educated by tumors and converted into TAMs, which can actually promote tumor progression and assist tumor cell metastasis. TAMs can also assist in local infiltration and distant dissemination of tumor cells by participating in lymphangiogenesis and angiogenesis. To a certain extent, TAMs can aggregate and even play a leading role in tumor cell metastasis. Previous studies have confirmed that depletion of TAMs is equivalent to turning off the switch on angiogenesis, and eliminating Tie2 TAMs can inhibit angiogenesis in mouse glioma (67,68).

Hypoxia is a common phenomenon in most solid tumors, as there are several novel blood vessels in the hypoxic area due to low oxygenation. Therefore, hypoxia is considered the primary driving force for angiogenesis. Research has revealed that hypoxic areas often accompany the aggregation of TAMs, which is primarily caused by hypoxia stimulating the production of a series of chemotactic and active factors, such as CCL-2, CXCL4 and VEGF, in the tumor and interstitial cells. In addition, TAMs can respond to hypoxia by upregulating the expression of HIF and downstream proangiogenic factors (69). Therefore, as the cells at the leading edge of the invading tumor, TAMs are also known as the cells that aggregate to form the premetastatic niche; thus, these cells can be used to identify the direction of tumor cell metastasis to blood and lymphatic vessels and assist in the prevention of metastasis.

The evidence that TAMs are directly involved in lymphangiogenesis is that TAM depletion can significantly weaken VEGFC and VEGFR3 signaling in LECs, weakening lymphatic vessel formation in early-stage tongue SCC (70,71). Hosono *et al* (72) found that in esophageal squamous cell carcinoma (ESCC), TAMs can release CXCL8 and bind to CXCR1/2 (known as CXCL8 receptors) of ESCC cell lines, promoting ESCC invasion by suppressing Akt and Erk1/2 phosphorylation. Neutralizing antibodies against CXCL8, CXCR1 and CXCR2 can inhibit these effects. Clinical analysis confirmed that CXCL8+ TAMs are associated with LNM in esophageal cancer. Chen *et al* (57) uncovered a new LNM model for CC in the hypoxic TME: ZEB1 increases CCL8 secretion and recruits TAMs to encapsulate the lymphatic vessel to form network centers, promoting CC LNM.

There are several studies on M2-like macrophages in the invasion and LNM of CC. Guo *et al* (73) found that the infiltration of CD68+ TAMs and CD163+ M2 TAMs is correlated with tumor progression. CD163+ M2 TAM infiltration is correlated with LNM and an advanced FIGO stage. Tan *et al* (74) found that activated T-cell nuclear factor 1+ TAMs, as the M2 TAM subtype, are significantly more abundant in CC tissues and can promote CC cell proliferation and metastasis by activating the c-Myc/PKM2 pathway. Jiang *et al* (75) found that during the progression from CIN I-III to stage I-IV CC, the levels of TAM aggregation and neovascularization in the TME increased synchronously, which fully confirmed that TAMs and tumor angiogenesis play a key role. Li *et al* (76) found that the number of M2-TAMs in CC was higher than that in the surrounding tissues, and the number of M2-TAMs in the diffusion infiltration pattern (DIP) was higher than that in a pushing border pattern (PBP), indicating a strong relationship between M2-TAMs and the invasive behavior of CC. The above research comprehensively showed that TAMs promote the LNM of CC. The identification of its regulatory mechanism not only provides a novel target for the development of therapies to counter metastasis but also provides a basis for selecting specific patient cohorts that may benefit from certain molecular-targeted drugs.

MDSCs. MDSCs are a group of heterogeneous immature myocytes that are blocked from maturing in cancer. They are one of the primary driving forces behind the immunosuppressive TME. According to phenotypic and morphological differences, these cells can be divided into two subgroups: Granulocytic MDSCs (G-MDSCs), which are similar to neutrophils, and monocytic MDSCs (Mo-MDSCs) (77). The increase in the number of MDSCs in CC patients was first confirmed in 2014 and the number of MDSCs, especially G-MDSCs, significantly increased in the tumor tissue of CC patients. MDSCs are important immune components in the TME and are considered to mediate the immunosuppression of tumor-bearing mice and cancer patients (78). In addition to enhancing immunosuppression, MDSCs have also been shown to enhance tumor progression by stimulating cancer cell invasion, metastasis, and tumor angiogenesis (79). In the TME of CC, tumor cells secrete various molecules to recruit MDSCs from immature myocytes, including granulocyte colony-stimulating factor (G-CSF) (80), IL-6 (81),

and highly expressed C-X-C chemokine receptor 2 (CXCR2) chemokines, such as CXCL1, CXCL2, CXCL3, CXCL5 and CXCL8 (82). Mo-MDSCs enhance the stemness of pancreatic cancer cells by producing IL-6 and subsequently activating STAT3 activator in cancer cells (83). Peng *et al* (84) recently showed that MDSCs enhance the dryness of breast cancer cells by producing IL-6 and nitric oxide and subsequently activating the STAT3 and Notch signaling pathways, respectively. Kuroda *et al* (85) found that G-CSF-induced MDSCs enhance the dryness of CC cells by producing prostaglandin E2 (PGE2). It was also demonstrated that the inhibition of MDSCs or PGE2 effectively inhibits the induction of CSCs and enhances the efficacy of cisplatin in CC. Ni *et al* (86) revealed that patients with high levels of METTL3 and CD33 expression in MDSCs in tumor tissues have a poor prognosis, and these phenotypes are independent risk factors for the prognosis of CC patients. Heeren *et al* (87) found that elevated MDSC levels increase CC LNM and weaken sensitivity to radiotherapy and chemotherapy. Rodríguez and Ochoa (88) further confirmed that MDSC-mediated niche formation before LNM can induce FDG uptake during FDG positron emission tomography/CT scans and leads to false-positive detection of an LNM. Using the HPV-mediated CC mouse model, researchers have demonstrated that MDSCs mediate immunosuppressive activity through IL-6/JAK/STAT3 signaling. The activation of STAT6 mediated by the proinflammatory cytokine IL-3 may be the reason for the expansion of MDSCs, which then accelerates tumor growth (89). In addition, MDSCs interact with B lymphocytes in the TME of CC through B-cell activating factor (BAFF) expressed on the surface of MDSCs. MDSCs induce B cells to differentiate into Breg cells by acting on BAFF receptors expressed in B cells. In addition, IL-10 secreted by Breg cells can promote STAT3 phosphorylation and activate MDSCs, thereby establishing a positive feedback loop. The continuous differentiation of Breg cells and the activation of MDSCs induce immunosuppressive states and lead to tumor immune escape in CC patients (90). MDSCs also stimulate tumor angiogenesis by secreting Bv8 (a proangiogenic molecule), which increases the expression of tumor G-CSF and MDSCs. CC patients have poor survival rates, and G-CSF-producing CC is sensitive to cisplatin after splenectomy or administration of anti-Gr-1 antibodies, leading to the depletion of MDSCs (91). In summary, MDSCs utilize multiple mechanisms to enhance the proliferation and metastasis of CC. After being recruited to the TME, they mainly exert strong immunosuppressive effects by inhibiting T-cell function.

Immune and inflammatory cells. During the immune escape process of tumors, regulatory T cells (Tregs) secrete large quantities of IL-10, TGF-β, and IL-35, which inhibits antigen presentation by dendritic cells and CD4 helper T-cell function, regulates the expression of inhibitory receptors and CD8-tumor-infiltrating lymphocyte (TIL) depletion-related transcriptome characteristics, promote T-cell depletion in tumors, downregulate antitumor immunity, and produce tumor-specific CD8 cytotoxic T lymphocytes (92). As the role of adaptive immune cells in the tumor microenvironment has been elucidated, the first treatment scheme that interferes with the function of T cells has been successfully approved by the U.S. FDA for antibody-based treatment of patients with advanced cancers, such as Nivolumab, Balstilimab and Zalifllimab. Wu *et al* (93) showed that a significant number of CD4+ CD25+ FOXP3+ Treg cells accumulate around tumor cells and that the proportion of FOXP3+ T cells in CC is higher than that in cervical intraepithelial neoplasia. Moreover, the proportion of FOXP3+ T cells in CC with LNM is significantly higher than that in CC without LNM. Nakamura *et al* (94) found that the higher the Foxp3+/CD4+ Treg cell ratio was, the greater the rate of LNM was. Foxp3+ Treg cells contact the immunoregulatory enzyme indoleamine 2,3-dioxygenase (IDO)+ APC. Foxp3+ Treg cells form a premetastatic microecology and establish a network with IDO to induce immune escape. Compared with that in the satellite lymph nodes without tumors, the proportion of Foxp3 Treg cells is significantly increased in lymph node metastases (94). There is a high incidence of LNMs at Foxp3 Treg cell aggregation sites. Foxp3+ Treg cells can directly contact IDO APCs in the context of LNM. In the metastatic microenvironment of CC, Treg cells first infiltrate and accumulate to form an immunosuppressive microenvironment to attract cancer cells to the site of metastasis. These studies provide evidence that the recruitment of Foxp3+ Tregs can promote cancer cell migration.

Previous studies have noted that based on flow cytometry analysis, CC patients with a low CD8 T-cell/Treg ratio, high Treg level, and high levels of PD-L1 and major histocompatibility complex, class II, DR (HLA)-DR myeloid cells are more likely to have LNMs; therefore, this environment can also be considered an immunosuppressive microenvironment. Heeren *et al* (95) found that delineated fields of Treg-associated immune suppression in anatomically colocalized tumor-draining lymph nodes primarily form a micro-transfer microenvironment to further integrate and expand to further regions, which provides a basis for early surgical intervention. In another study by the same lab, it was found that a higher number of CD8+ T cells significantly reduced the frequency of CD4+ cells, increased the expression of the memory marker CD45RO and activation markers [PD-1, inducible T cell costimulator, HLA-DR and cytotoxic T-lymphocyte-associated protein (CTLA-4)], and significantly promoted LNM. Furthermore, they discovered that in metastatic lymphoid tissue, the proportions of the FoxP3+ Tregs and regulatory antigen-presenting cells (APCs) [PD-L1+ CD11c(hi), CD14+], APC/myeloid suppressor cell subpopulations increased significantly. After treatment with different Toll-like receptor ligands, the expression of IFN-γ in metastatic lymphoid tissue significantly decreased, but IL-10, IL-6, and TNF-α expression significantly increased (87). Wang *et al* (96) used 18F fluorodeoxyglucose microPET/CT and bioluminescence imaging analysis of mouse neck xenograft tumors to confirm that PD-L1 overexpression promoted tumor glucose uptake by activating the ITGB4/SNAI1/SIRT3 signaling pathway, ultimately leading to LNM. At present, it is suggested that targeting PD-L1 and CTLA4 is a potential approach for the treatment of LNM in CC.

Research on the relationship between tumors and inflammation is developing constantly. Tumor-induced inflammation can result in DNA damage and micrometastases. Systemic inflammatory responses can aggravate malnutrition in patients. Inflammatory indicators have certain reference values in

the diagnosis and prognosis of various malignant tumors, and research has confirmed that inflammation may promote tumor progression (97). Zhang *et al* (98) found that the neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) were valuable for predicting LNM in gastric cancer. The NLR is superior to the PLR in predicting the gastric cancer survival rate. Ayhan *et al* (99) found that a high NLR and PLR was significantly correlated with tumor volume (>2 cm) and deep muscle interstitial infiltration. A high monocyte-to-lymphocyte ratio was significantly correlated with abdominal aortic LNM, pelvic LNM, and locally advanced CC (IB3-IIIC2). Lee *et al* (100) confirmed that an NLR ≥3.1 was indicative of a shift in the immune response from tumor suppression to cancer promotion, often accompanied by radiation resistance, rapid tumor progression, and LNM, which is associated with a poor prognosis in CC patients. The use of inflammatory indices in predicting the clinical outcomes of patients with lymphatic metastasis of CC is deserved of further attention in light of the convenient and low-cost means of access to the data.

4. Therapeutic strategies for targeting the TME of CC

The standard method of treatment for advanced CC includes radiotherapy and chemotherapy; however, the patient survival rate is very low (101). In recent years, the gradual promotion and application of immunotherapy has provided renewed hope. The role of the TME in tumor metastasis has been confirmed; cancer cells can recruit Tregs, downregulate cancer cell surface antigens, induce T-cell apoptosis, secrete immunosuppressive factors, and evade immune surveillance and attack, eventually forming the immunosuppressive TME on which they rely for survival (102). The primary purpose of immunotherapy is to reactivate the antitumor immune response or reshape the immunosuppressive microenvironment. The US Food and Drug Administration (FDA) has approved three immunotherapy-based drugs for the treatment of CC: Pembrolizumab, Tisotumab Vedotin and Nivolumab. Other immunotherapeutic strategies for the TME are still in the exploratory stage, and the progress of TME treatment strategies for CC is briefly summarized below.

Targeting hypoxia. Hypoxia increases the risk of local invasion, metastasis, and treatment failure in CC. Hypoxia represents an attractive therapeutic target, and a number of strategies have been researched. The known studies on hypoxia-targeted strategies are as follows: i) Increasing intratumoral oxygen: A phase II clinical trial of 139 patients with locally advanced CC concluded that the addition of carbogen and nicotinamide hypoxia modification to standard therapy was feasible and safe (103). ii) Decreasing tumor oxygen consumption: Metformin, an antidiabetic agent, reduces cancer incidence in patients with diabetes (104). Subsequent experiments highlight a complex interplay with hypoxia-associated molecular pathways, possibly through the inhibition of the mammalian target of the rapamycin-HIF-1α axis (105). Two phase II randomized clinical trials, NCT02394652 and NCT04275713, are currently investigating the use of metformin as a hypoxia-modifying therapy for locally advanced CCs. These trials will assess metformin-induced changes in tumor hypoxia using imaging and gene expression biomarkers (3). Hypoxia-specific radiosensitizers and cytotoxins: There are several classes of hypoxia-specific cytotoxins. Quinone-based agents selectively activate hypoxia through a reductive mechanism and induce DNA alkylation-mediated cytotoxicity. Sharma *et al* (106) performed a study with 160 patients with locally advanced squamous cell carcinoma of the uterine cervix that participated in a multicenter phase III trial that randomized participants to receive radiotherapy alone or radiotherapy with concomitant mitomycin C. Despite improved 4-year disease-free survival rates in the intervention group, the study failed to show a significant benefit in overall survival or local recurrence rates. Discovered ~35 years ago by Zeman *et al* (107) and Brown (108), tirapazamine was the first purely hypoxic cytotoxin and is one of the most advanced bioreductive drugs in clinical trials. The best-known aromatic N-oxide is used as an anticancer drug that undergoes enzymatic one-electron reduction and converts to an electron-donating mono-N-oxide metabolite (tirapazamine radical). Murine model experiments showed considerably more tumor cell death was observed when tirapazamine was combined with radiotherapy or cisplatin chemotherapy compared with monotherapy (108), but unfortunately, the follow-up clinical trial of DiSilvestro *et al* (109) failed to show a clinically meaningful result. iv) Hyperthermia: A strategy that encompasses a variety of hypoxia-targeting mechanisms is hyperthermia. It is assumed to improve oxygenation by causing vasodilatation, direct cellular damage, immune-mediated killing of tumor cells, and inhibition of DNA repair. In CC, hyperthermia has been used to sensitize tumors to radiotherapy, and the evidence suggests that combining radiotherapy with hyperthermia results in improved locoregional control when compared with using radiotherapy alone (77 vs. 52%) (38). Effectively decreasing hypoxia would clearly improve the response to therapy and reduce the likelihood of metastatic spread in CC.

Targeting immune checkpoint molecules. The tumor microenvironment plays a key role in tumor metastasis. Cancer cells can recruit Tregs, downregulate tumor antigen expression, induce T-cell tolerance and/or apoptosis, and generate immunosuppressive cytokines to stimulate immunosuppressive immune checkpoints, which leads to a unique and highly immunosuppressive tumor microenvironment (TME) (102). To overcome these immunosuppressive conditions, immune checkpoints may be modulated by either agonist or antagonist monoclonal antibodies used to enhance T-cell activation and eliminate inhibition of T-cell activation, respectively, to reactivate T cells to attack tumors (110). At present, immune checkpoint inhibitors in CC primarily include the following: i) Programmed death ligand 1 (PD-L1): PD-L1 is an immunomodulator that is expressed on antigen-presenting cells (APCs) and 20-50% of human cancer cells. Tumor-induced PD-L1 inhibits T-cell function and induces immune tolerance but also induces T-cell apoptosis. By contrast, PD-L1 induces expansion registration of T cells. Therefore, blocking this ligand on tumor cells and APCs can improve tumor defense, and T cells with anticancer properties can restore their effector functions (111). ii) Anti-CTLA4 antibody: Under physiological conditions, T cells are stimulated by CD28, and CD28 interacts with B7-1 and B7-2 on dendritic cells. In addition to

the 'key' CD28, T cells also express CTLA4, which can be regarded as 'key off'. CTLA4 acts as a symbiotic factor on activated T cells to regulate their immune response (112). The application of immune checkpoint inhibitor targeting in CC is summarized in Table I.

Early data suggested that immune cells, in particular CD8+ T cells, play a key role in tumor cell death within a radiation field (119). Radiation therapy causes migration of dendritic cells and cross-penetration of tumor antigens, which can result in T-cell activation and proliferation. Furthermore, radiation therapy increases the density of TILs within a tumor, likely via extravasation of TILs within the vasculature of tumors and chemokine activation (120). It is known that radiation therapy alters the T-cell receptor repertoire of peripheral T-cell clones (121). Thus, there is a strong rationale for the combination of radiation with immune checkpoint blockade. There are limited data surrounding the optimal dose and fractionation needed to provoke an ideal immune response when combining immunotherapy with radiation in CC. Sequencing of CTLA-4 blockade with immunotherapy in preclinical models demonstrates that when anti-CTLA-4 is delivered prior to radiotherapy, there is increased efficacy compared to delivery after radiotherapy (122). Studies have also demonstrated that radiotherapy increases PD-L1 expression, which may act as a negative feedback mechanism preventing T-cell-mediated tumor rejection (123). Radiotherapy and chemotherapy combined with PD-1 and CTLA-4 immune checkpoint blockades provide a more effective scheme than monotherapy for the treatment of advanced and recurrent cervical cancer.

Targeting suppressive immune cells. Suppressive immune cells, such as Tregs, MDSCs, and type 2 macrophages, form an immunosuppressive microenvironment to assist tumor cell escape from immune surveillance. Research has shown that practical CXCR2 antagonist therapy can weaken the proliferation and migration of CC cells (124). In addition, the method of targeting the CSF-1/CSF-1R axis of TAMs is being assessed in a mouse model. CSF-1R inhibition weakened the turnover rate of TAMs and increased the number of CD8 T cells infiltrating tumor tissue (125). The antitumor effect of anti-PD-1 therapy is enhanced by inhibiting CXCR2, the primary chemokine receptor for MDSC recruitment in human pancreatic cancer (126). However, these studies are still limited to *in vitro* and *in vivo* experiments, although they provide novel ideas for future clinical trials. In addition, metabolites targeting suppressive immune cells, such as Arg-1 and IDO, are also novel avenues for targeting suppressive immune cells. The arginase inhibitor INCB001158 is being used to treat metastatic solid tumors (NCT02903914). Treatment of IL-6 knockout mice with IDO inhibitors has been proven to inhibit the expression of IDO. In addition, combination therapy with therapeutic vaccines leads to a decrease in polymorphonuclear MDSCs and Treg cells in tumors, supporting IL-6 and IDO as immunometabolic adjuvants for immunotherapy against CC (127). Combination therapy targeting inhibitory immune cells and metabolites within the TME represents a new strategy for antitumor therapy.

Anti-lymphangiogenesis and anti-inflammatory therapy. Since lymphatic vessels and lymphatic remodeling play a key role in lymphatic metastasis, targeting lymphatic vessels is the key to the treatment of metastatic CC. The VEGF-C/VEGFR-3 signaling axis induces tumor lymphatic vessel formation. In an experimental model, blocking VEGF-C/VEGFR-3 has been proven to reduce tumor lymphatic vessel formation and metastasis (128). However, this study has not yet entered a clinical trial stage. Inflammation plays a certain role in tumor metastasis. Nonsteroidal anti-inflammatory drugs combined with chemotherapy and radiotherapy can increase the sensitivity of patients with locally advanced cervical cancer. Studies have confirmed that blocking the inflammatory signaling pathway (COX/PGE2) and regulating the immune response against HPV and targeting the virus are the best choices for antitumor treatment of cervical cancer (129). The interaction of various cells in the TME is very complex, and the effect of any single therapy is limited. Combination therapy may provide a breakthrough for improving the prognosis of patients with recurrent and metastatic CC in the future.

5. Conclusions and future perspectives

Lymphatic metastasis is a key factor affecting the prognosis of patients with cervical cancer. CAFs, TAMs, and immune and inflammatory cells (primarily T cells and neutrophils) in the tumor microenvironment promote lymphatic metastasis by releasing a series of cytokines (such as VEGF-A/C/D and TGF-β) to induce tumor cell EMT, lymphatic vessel proliferation, and immune evasion, which ultimately leads to lymphatic metastasis. The above effects are enhanced under hypoxic conditions through hypoxia-related signaling pathways and transcription factors (such as HIFs). Although progress has been made in clinical trials on hypoxia-targeting strategies, and PD-1 and CTLA-4 immune checkpoint blockades in advanced CC, there are few clinical trials and drugs that specifically target markers for predicting and treating lymphatic metastasis in CC (130). A few clinical trials have shown that simultaneous radiotherapy combined with immunotherapy is more effective than monotherapy, but the specific mechanism remains unclear, and it is meaningful to expand the population to further study the mechanism of action to guide clinical treatment. The tumor heterogeneity of individual CC patients increases the complexity of treatment and leads to differences in the involvement of factors related to lymphatic metastasis among patients. Hypoxia can allow tumor cells to appear dormant, increase the difficulty of treatment, and recruit more TAMs as a lymphatic angiogenesis switch. TAMs directly participate in lymphatic angiogenesis. CAFs damage the lymphatic endothelial barrier and destroy the integrity of the lymphatic endothelium, and immune-inflammatory cells to create an immunosuppressive microenvironment. These complex and orderly steps involved in tumor microenvironment formation eventually leading to LNM. However, the mechanism is still unclear. There remain several aspects that need to be studied and explored in the future to understand and reduce the incidence of lymphatic metastasis of CC and improve the survival rate. Development of individualized treatments based on the tumor microenvironment is an important direction that is expected to be an important strategy for treating lymphatic metastasis of CC in the future.

Table I. Summary of clinical trials targeting immune checkpoint molecules in cervical cancer.

First author, year	NCT Number/ phase of clinical trial	Immunotherapeutic regimen	Additional therapy	Patient population (n)	Trial status/clinical efficacy	(Refs.)
Naumann, 2019	NCT02488759/Phase 2	Nivolumab (anti-PD-1 antibody)		Recurrent/metastatic cervical, vaginal or vulvar cancer (24 participants)	Completed/ORR, 26.3%; MOS, 21.9 months	(113)
O'Malley, 2022	NCT03495882/Phase 2	Balstilimab (anti-PD-1 antibody) and Zalifrelimab (anti-CTLA-4 antibody)		Recurrent and/or metastatic cervical cancer (155 participants)	Completed/ORR, 25.6%; PDL-1+, 32.8%; PDL-1(-): 9.1%.	(114)
Santin, 2020	NCT02257528/Phase 2	Nivolumab (anti-PD-1 antibody)		Persistent, recurrent, metastatic cervical cancer (26 participants)	Completed/PFS and OS at 6 months were 16 and 78.4%, respectively.	(115)
Frenel, 2017	NCT02054806/Phase 1	Pembrolizumab (anti-PD-1 antibody)		Advanced cervical cancer (42 participants)	Completed/ORR, 17%	(116)
Chung, 2019	NCT02628067/Phase 2	Pembrolizumab (anti-PD-1 antibody)		Advanced cervical cancer (46 participants)	Completed/ORR, 12.2%; PD-L1+ ORR, 14.6%	(117)
De Jaeghere, 2023	NCT03192059/Phase 2	Pembrolizumab (anti-PD-1 antibody)	Radiotherapy, vitamin D, Aspirin, Lansoprazole, Cyclophosphamide, and Curcumin	Refractory or persistent endometrial, cervical, or uterine cancer patients (43 participants)	Completed/ORR, 11.1%; MOS, 39.6 weeks	(118)

ORR, objective response rate; MOS, median overall survival; NCT, National Clinical Trial; HPV, human papillomavirus; PD-1, programmed cell death 1; PD-L1, PD1 ligand 1; CTLA, cytotoxic T-lymphocyte-associated protein; PFS, progression-free survival.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

LFW and JC conceived the concept of the review and revised the manuscript. LFW, SYY, YT, and WHL drafted the manuscript. LFW and WHL prepared the figures. Data authentication is not applicable. All authors have read and approved the final manuscript.

Ethical approval and consent to participate

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Patient consent for publication

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Competing interests

The authors declare that they have no competing interests.

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